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Liquid Chromatography of Synthetic Polymers under Limiting Conditions of Insolubility III

Application of Monolithic Columns

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Summary Performance was evaluated of silica based commercial monolithic rod-like columns in liquid chromatography of synthetic polymers under limiting conditions of enthalpic interactions (LC LC). LC LC employs the barrier effect of the pore permeating and therefore slowly eluting small molecules toward the pore excluded, fast eluting macromolecules. Phase separation (precipitation) barrier action was applied in present study. The barrier was created either by the narrow pulse of an appropriate nonsolvent injected into the column just before the sample solution (LC LC of insolubility – LC LCI) or by the eluent itself. In the latter case, the polymer sample was dissolved and injected in a good solvent (LC LC of solubility – LC LCS). In LC LCI, polymer species cannot break thru the nonsolvent zone while in LC LCS they cannot enter eluent, which is their precipitant. Therefore, polymer species keep moving in the zone of their original solvent. Macromolecules eluting under the LC LC mechanism leave the column in the retention volume (V_R) roughly corresponding to V_R of the low molar mass substances and can be efficiently separated from the polymer species non-hindered by the barrier action. The known advantages of monoliths were confirmed. From the point of view of LC LCI and LC LCS the most important quality of monolithic columns represents their excellent permeability, which allows both working at high flow rates and injecting very high (in the range of 5%) sample concentrations. Monolithic column tolerate also extremely high molar mass samples ($M > 10{,}000$ kg · mol^{-1}). On the other hand, the mesopores (separation pores) of the tested monoliths exhibited rather small volume and wide size distribution. These shortcomings partially impair the permeability advantage of monoliths because in order to obtain high LC LC separation selectivity a tandem of several monolithic columns must be applied. Presence of large mesopores also reduces applicability of monolithic columns for molar masses below about 50 kg · mol^{-1} because V_Rs of polymers eluted behind the barrier are similar to that of freely eluting species. The non- negligible break-thru phenomenon was observed for the very high polymer molar masses largely eluting behind the barrier. It is assumed that the fraction of very large mesopores present in the monoliths or association/microphase separation of macromolecules may be responsible for this phenomenon. This is why the presently marketed SiO_2 monolithic columns are mainly suitable for the fast purification of the LC LC eluting macromolecules from the polymeric admixtures non-hindered by the barrier-forming liquid. Still, monolithic columns have large potential in the LC LCI and LC LCS procedures provided size (effective diameter) of the mesopores can be reduced and their volume increased.

Keywords: limiting conditions of enthalpic interactions; liquid chromatography; monolithic columns; phase separation retention mechanism; synthetic polymers

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Introduction

The term ''Liquid chromatography under limiting conditions'' (LC LC) designates a group of techniques utilizing *the barrier principle* for separation of macromolecules.[1–14] LC LC columns contain porous fillings, from which polymer species are partially or fully excluded. As result, macromolecules are eluted from such columns within low retention volumes. On the contrary, the small molecules of mobile phase or the appropriate low molecular auxiliary substances freely permeate the filling pores and therefore their elution rate is low. Applying suitable enthalpic retention mechanism, the fast progression of polymer species is selectively blocked by the slowly moving, ''lazy'' barrier.

Barriers can be formed by the mobile phases preventing elution of selected macromolecules.[2,4,7,13] In this case, polymer sample is dissolved in a solvent, which promotes its elution. Polymer species subject to enthalpic interactions stay confined within the slowly moving zone of their original solvent while the unretained species hurry along the column and elute in the exclusion mechanism.

An alternative method for creation of a barrier employs injection of a retention promoting substance together[5,11] or just before[9,12,13] the sample zone. Evidently, the former approach cannot be applied for the phase separation retention mechanism. In any case, the unretained macromolecules break-out or -through, respectively and elute in the size exclusion chromatography (SEC) mode while fast elution of polymer species subject to enthalpic interactions is hampered by the slowly eluting narrow barrier zone.

Applying the above arrangements, macromolecules of different nature can be easily and efficiently separated with help of appropriate barriers. So far, three enthalpic retention mechanisms of synthetic polymers were applied for the barrier action within the particulate column packings

- adsorption on the packing *surface*[3–11] promoted by the chromatographically *weak solvents*
- enthalpic partition (absorption) in favor of alkyl bonded phase *volume*[14] raised by the thermodynamically *poor solvents* for macromolecules
- phase separation (precipitation) by the *nonsolvents* for macromolecules.[2,4,12,13]

Correspondingly, six different LC LC procedures have been established, three with the continuous (eluent) barriers and three with the local barriers in the form of narrow pulses.

Adsorption of samples is as rule controlled with the elution *strength* of either the mobile phase or the local barrier while the thermodynamic *quality* of the latter two constituents of chromatographic system toward macromolecules affects enthalpic partition and phase separation of polymer species. Interactive column packings are applied in case of adsorption or enthalpic partition based LC LC procedures while also the non-active column packings work well when utilizing phase separation retention mechanism. It has been shown that two retention mechanisms can be simultaneously operative in certain chromatographic systems containing macromolecules.[4] Existence of such hybrid barrier action may complicate the separation control.

Recently, the LC LC approach was compared with liquid chromatography under critical conditions of enthalpic interactions (LC CC),[11] the role of experimental variables in various LC LC procedures was elucidated,[7,8,13] and the applications of the LC LC methods for separation of selected polymer blends was demonstrated.[7–9,12,13] Both bare and alkyl bonded silica gels and poly(styrene-co-divinylbenzene) based particulate column packings with various pore sizes were employed. The most promising appeared the adsorption retention mechanism both with the continuous barrier of eluent (LC under Limiting Conditions of Adsorption – LC LCA) and with the local barrier of an

adsorption promoting liquid – *an adsorli* (LC under Limiting Conditions of Desorption – LC LCD). Efficient separations were attained also by the application of the phase separation mechanism with the local barrier of a nonsolvent (LC under Limiting Conditions of Insolubility – LC LCI). The LC LCA, LC LCD and LC LCI methods afforded the extraordinary narrow, focused polymer peaks because macromolecules accumulated on the barrier edge. On the contrary, the LC LC procedures employing phase separation retention mechanism with a nonsolvent as mobile phase (Liquid Chromatography under Limiting Conditions of Solubility, LC LCS) and also the LC LC separations applying a poor solvent either as the mobile phase or as the local barrier in combination with the alkyl bonded column packing (Liquid Chromatography under Limiting Conditions of Partition – LC LCP and Limiting Conditions of Unpartition – LC LCU, respectively) produced broadened and sometimes ill shaped peaks. This may be caused by the slow establishment of equilibrium in the latter chromatographic systems.

It was of interest to test the applicability of monolithic columns for the LC LC separations of macromolecules. As known[15] monoliths exhibit the decreased resistance against the flow of both eluents and (viscous) samples. This could allow high speed LC LC separations, and/or fractionations of the complex systems at increased concentration for preparative purposes or sample purification, as well as for identification and molecular characterization of minor components (<1%) in the multi-component polymers. Phase separation retention mechanism was applied in present study that is the performance was studied of monolithic columns in the LC LCI and LC LCS procedures.

Monolithic Columns in High Performance Liquid Chromatography

Substitution of conventional liquid chromatographic columns containing a bed of particulate packings by a single piece of porous material, a monolith, was apparently attempted by several researchers. For example, Čoupek[16] applied rods of homogeneously crosslinked narrow pore poly(hydroxyethyl methacrylate-co-ethyleneglycol dimethacrylate) polymers for the SEC separation of synthetic and biological macromolecules already in the beginning of seventies of the last century. His experiments were not successful because the columns were easily blocked with large polymer species. Hjertén et al.[17–19] solved this problem applying array of compressed semi rigid particles of crosslinked poly(acryl amide). In this way, interparticle volume was reduced but the flow of mobile phase among particles was not completely blocked. In their pioneering work, Hjertén et al.[20] eventually demonstrated also creation of continuous gel ''plugs'' from copolymers of acrylic acid and N,N′-methylenebisacrylamide, which were sufficiently both permeable and rigid to allow high flow-rates of eluents.

The subsequent research in several laboratories[21–42] has shown the advantages of strong, non-compressible monoliths containing two kinds of pores

a) large flow-thru channels called also *macropores* though probably the term *gigapores* should be preferred in this case. Macropores allow almost free flow of eluent and sample molecules along the monolith
b) *mesopores,* which provide surface (or bonded phase volume) necessary for the successful separation.

Recently, numerous monolithic columns were prepared on the base of various organic polymers both in the form of thick rod-like plugs or disc, and also within capillaries.[21–30] Alternative material for preparation of monolithic column is silica gel.[15,31–35] The silica rods are encapsulated in the appropriate plastic containers or they are created directly in capillaries. The general issues of monolithic columns were analyzed in several review papers.[15,36–40]

Most above authors studied physical and chromatographic properties of monolithic

columns. Inverse SEC was used to estimate volumes and sizes of both macro- and meso-pores.[39–41] There are some doubts about utilization of inverse SEC for quantitative characterization of pore size distribution in the solid systems. For example, large discrepancy was found between mercury porometry and size exclusion chromatography data for various inorganic porous materials.[43] Still, inverse SEC can furnish valuable information on total both external and internal porosities of packed and monolithic columns.[39–41] The above studies confirmed bimodal pore structure of the rod-like monoliths with the flow-thru macropores and mesopores. The total porosity of the thick rod-like silica monoliths has been shown to be about two times higher compared to the HPLC columns packed with porous particles. About 80–85% of total porosity was due to flow-thru pores and only up to about 20% due to mesopores. Thus the internal porosity that is the volume of mesopores of the presently available rod-like silica monoliths is up to 70% lower than in particulate silica columns. This is an important shortage of monoliths from the point of view of the SEC, LC CC and LC LC applications. Volume of mesopores (separation pores) is important for both the SEC and the LC LC separation selectivities. For example, in LC LC the volume of mesopores is reflected in the difference between retention volumes of excluded macromolecules and barrier forming small molecules. This difference together with the size of mesopores determines the separation selectivity of LC LC. It is of interest to point-out that the effective volume of separation mesopores seems to be greater in the capillary monoliths compared to the rod-like monoliths.[41,42,44]

The important feature of the monolithic HPLC columns is their increased permeability. Dependences of pressure on flow rate are linear even at very high eluent flow rates. Back-pressure of monoliths is low and for commercial silica monoliths with macropores in the range of 2 μm it roughly corresponds with that of columns packed with 11 μm beads.[26] This could allow applications of monoliths at reasonably high flow rates even for concentrated polymer samples.

Evidently, the gross pore size distribution of silica rod-like monoliths is discontinuous and includes pores ranging from few nanometers up to few micrometers. The average pore size of mesopores in commercial silica monoliths from Merck is about 13 nm and size of macropores is about 2 μm.[15] On the other hand, it is likely that the capillary silica monoliths prepared by Ute et al.[42] contain mesopores exhibiting broad size distribution, which enables their SEC application over wide range of polymer molar masses. However, this property is unwanted in the LC LC separations where the macromolecules as small as possible should be fully excluded from the pores of the column filling.

Another important feature of monolithic HPLC column is their remarkable efficiency at high linear flow velocities, which results in the flat van Deemter plots.[15] This property of monoliths allows their employment in the high-speed HPLC and it may be important also for the fast polymer separations though the LC LC methods usually produce narrow, focused polymer peaks.[7–9,11,13]

Experimental Part

Apparatus

A simple high performance liquid chromatographic (HPLC) assembly was used. The pumping system was Knauer 64 (Knauer Co., Berlin, Germany). The detectors were either a differential refractometer, Erma ERC, Model 7515A (ERC Inc., Tokyo, Japan) or an evaporative light scattering detector (ELSD) Model DDL-21 (Eurosep, Cergy-St. Christophe, France). The data were collected and processed with Baseline software from Waters, USA. The sample solutions and the nonsolvent pulses were injected by means of a tandem of two six-port two-way high pressure valves from

Rheodyne, Cotati, CA, USA - Model 7725i for polymer solution and Model 7125i for the nonsolvent barrier zone in case of LC LCI.[13] The valves were provided with the loops of different volumes. The sample loop volumes ranged from 20 to 200 μL, and the volumes of nonsolvent loops varied from 50 to 500 μL. Unless otherwise stated the flow rate was 1 $mL \cdot min^{-1}$.

In all experiments, entire loop volumes were injected. The valve containing nonsolvent was operated as first. Injection of polymer solution followed within one second. The electrical "start" impuls device was activated simultaneously with the nonsolvent valve. The columns were kept at $30\,^{\circ}C \pm 0{,}1\,^{\circ}C$ in an air column oven (Chroma, Graz, Austria), which was connected with a water thermostat, Model RM6 (Lauda, Königshofen, Germany). The pumping system was not thermostated, however, two capillaries of about 2 m total length and with 0.5 mm diameter were inserted between pump and injection valves. These capillaries were kept in the water bath and in the column thermostat in order to preheat eluent. The pressure gauge was purchased from Institute of Chemical Processes, Academy of Sciences of Czech Republic, Prague. Most measurements were done twice and results were averaged. Repeatability of measurements was better than 4%.

One bare silica based monolith Chromolith Performance SI 100×4.6 mm and two columns Chromolith SI were employed. The former column was a gift from Merck, Darmstadt, Germany while Chromolith SI columns were supplied by Merck Slovakia, Bratislava. In most experiments two or three monolithic columns were connected in series.

Molar mass values of broad polystyrene, poly(methyl methacrylate), and poly(vinyl acetate), were determined by size exclusion chromatography (SEC) in tetrahydrofuran employing Model 510 pump from Waters, Model 7125i injection valve from Rheodyne, linear AM gel column 7.8×300 mm from American Polymer Standards Corporation (Mentor, OH, USA), Model 410 refractive index detector from Waters and DAWN DSP multi-angle light scattering detector from Wyatt Corporation (Santa Barbara, CA, USA). Data were processed by software ASTRA from Wyatt and Clarity from DataApex (Prague, Czech Republic).

Materials

Polymer probes of various polarities were polystyrenes (PS), poly(methyl methacrylate)s (PMMA), and poly(vinyl acetate)s (PVAC).

Narrow molar mass distribution PS standards with weight average molar masses $\overline{M}_w$ from 0.67 to 2,000 $kg \cdot mol^{-1}$ were purchased from Pressure Chemicals (Pittsburgh, PA, USA). Ultra high molar mass polystyrenes with $\overline{M}_w$ 20,600 and 30,000 $kg \cdot mol^{-1}$ were products of Tosoh (Tokyo, Japan) and Polysciences (Warrington, PA, USA), respectively. Broad molar mass PS was the product of BASF Ludwigshafen, Germany. Its $\overline{M}_w$ was 180 $kg \cdot mol^{-1}$ and $\overline{M}_n$ 67 $kg \cdot mol^{-1}$. Poly(methyl methacrylate)s of medium molar mass distribution with weight averages of molar mass from 16 to 613 $kg \cdot mol^{-1}$ were gift of Dr. W. Wunderlich of Röhm, Darmstadt, Germany. Broad molar mass distribution sample of PVAC with $\overline{M}_w = 260$ $kg \cdot mol^{-1}$ and $\overline{M}_n = 95$ $kg \cdot mol^{-1}$ was a product of Duslo, Šaľa, Slovakia.

Eluent components were liquids exhibiting various both solvent strength considering bare silica gel and thermodynamic quality for polymers under study. These were toluene, acetonitrile (ACN), tetrahydrofuran (THF) and methanol. Toluene and methanol of analytical grade were obtained from Slavus (Bratislava, Slovakia), and THF of analytical grade from POCH (Gliwice, Poland). ACN of analytical grade was purchased from Merck, Darmstadt, Germany. All above solvents were distilled immediately before use. THF was stabilized with 0.1 wt. % of di-t-butyl p-cresol.

The monoliths were incorporated in the PEEK tubing. This reduced choice of eluents because for example tetrahydrofuran

may attack PEEK material and should be used either in a mixture with other solvents or only as a narrow pulse. Surprisingly, Guiochon et al.[39,44] used pure THF eluent in their studies of PEEK encapsulated monoliths from Merck without problems. THF possesses several valuable physical properties and it is the most common eluent or eluent component in SEC. THF is a medium strength solvent exhibiting moderate desorbing properties. Toluene is a low strength, low polarity solvent. It promotes full adsorption of PMMA within silica gel,[45] it is a typical *adsorli* for this polymer. THF and toluene prevent adsorption of PS on bare silica gel. They are thermodynamically good solvents for PS and PMMA while methanol is a rather efficient non-solvent for the both latter polymers. However, methanol dissolves PVAC. ACN exhibits solvent strength similar to THF but possesses much lower ability to dissolve synthetic polymers. ACN is a theta solvent for PMMA at about 34 °C.[46] Below its boiling point ACN does not dissolve PS. Both the solvent quality and the solvent strength of eluents and barriers were adjusted by mixing above liquids. The appropriate compositions of most mixtures were determined in previous studies.[12,13]

Results and Discussion

Flow Properties of Monoliths Used in Present Study

The flow resistance of system was tested with the neat toluene eluent and/or with the toluene solutions of PS. A precise pressure gauge was installed into the system. Flow rates were successively changed from 0.1 to 3 $mL \cdot min^{-1}$. The dependences of the flow rate on pressure were monitored. They were well linear in all experiments. Pressure reached 2.0 MPa at the flow rate of 3 $mL \cdot min^{-1}$ within the preheating capillaries but without columns and injection valves. The flow resistance of the system after adding a tandem of two monoliths, connecting capillaries and valves at the same flow rate was 7.4 MPa. The same experiment was repeated also with a tandem of three monolithic columns. This time, the maximum measured pressure was 9.5 MPa at 3 $mL \cdot min^{-1}$. Evidently, the monolithic columns exhibited remarkably low flow resistance. Next, various concentrations of broad PS were injected into the tandems of two and three monoliths from a 100 μL loop. Upon injection of a very high concentration (0.1 $g \cdot mL^{-1}$) viscous polymer solution pressure increase was only 1.5 and 2.1 MPa at the flow rate of 1 $mL \cdot min^{-1}$ for two or three monoliths, respectively. This is about one tenth of the pressure rise caused by viscous polymer solutions in the 250 mm column containing 10 μm particulate packings. In this way, the anticipated suitability of monoliths for fast elution of samples, as well as for viscous polymer solutions was confirmed.

Exclusion Properties of Monoliths

The dependences log M vs. V_R, for polystyrene standards in toluene are shown in Figure 1 for a tandem of two and three monolithic columns. M is the most abundant molar mass of particular polymer and V_R is retention volume at the peak apex.

Polystyrenes were injected in the neat toluene either directly into columns or via the injection valve for barrier liquid situated between the sample injection valve and the column so that polymer solutions passed thru the 100 μL barrier loop filled with eluent. The log M vs. V_R plots for particular single monolithic columns were almost identical (results not shown). This indicates good reproducibility of monolithic column production. All dependences of log M vs. V_R are rather non-linear while the shift in V_R observed with the tandem of two monoliths roughly corresponds to the volume of barrier loop, connecting capillaries and volume of channels within the barrier valve. For comparison, also the log M vs. V_R dependence is shown in Figure 1 for the PS standards in toluene obtained with the particulate 10 nm pore size column packing Kromasil 100 from Eka Chemicals, Bohus, Sweden. The latter dependence was

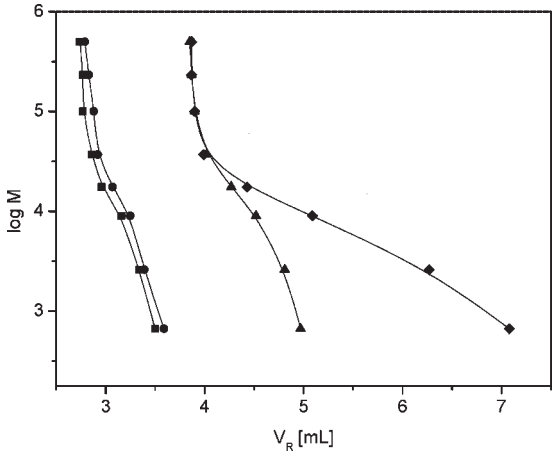

Figure 1.
The log M vs. V_R dependences for polystyrenes in toluene. Tandem of two monoliths: (■) sample injected directly into column; (●) sample injected thru the barrier loop filled with eluent. (▲) Tandem of three monoliths. For comparison also the values are shown, (◆) obtained with the Kromasil 100A particulate column packing. M data are in $g \cdot mol^{-1}$.

normalized to the retention volume of PS with molar mass 10 $kg \cdot mol^{-1}$ obtained with the three monoliths. The results displayed indicate that the effective size and size distribution of major fraction of mesopores (separation pores) in monoliths roughly correspond to 10 nm (100 Å) particulate silica gels. Still, it seems that the separation range of monoliths extends to higher molar masses because the log M vs. V_R dependences are not vertical even for high polymer molar masses. The shape of log M vs. V_R plots for large macromolecules can be explained by the effect of hydrodynamic separation mechanism[47] within the flow-thru macropores. The latter macropores occupy about 70% of total volume of the tested monoliths. In agreement with literature, the volume of separation pores represents less than 20% of the total volume of monoliths. This is only one third of the internal pore volume of particulate silica gel HPLC column packings, where the interparticulate volume is about 35–40 vol. %, the matrix occupies about 15 vol. % and 50 to 45 vol. % remains for separation pores. This result is similar to observation made by Guiochon et al.[39] with the rod-like monoliths, which were modified with the C-18 alkyl groups. The C-18 groups decrease effective pore size of 10 nm silica gel by about 50%.[48]

Both the low volume of separation pores and the nonlinearity of log M vs. V_R dependence of monoliths are disadvantageous when compared to good particulate column packings. Another limitation of present monoliths in light of the LC LC application is the relatively large size of their separation mesopores. Macromolecules with the molar mass below about 50 $kg \cdot mol^{-1}$ are only partially excluded from the mesopores of monoliths and their retention volumes may approach the retention volume of macromolecules eluting behind the barrier. Due to above properties of presently available commercial monolithic columns, efficient LC LC separation of polymer samples or their fractions with molar masses below about 50 $kg \cdot mol^{-1}$ will be limited. Moreover, a fraction of sample may tend to break-through the nonsolvent barrier in the columns packed with the wide pore material.[13]

Liquid Chromatography Under Limiting Conditions of Insolubility

Toluene/methanol 85/15 wt./wt. mixture was used as eluent in the preceding studies.[12,13] The barrier was either neat

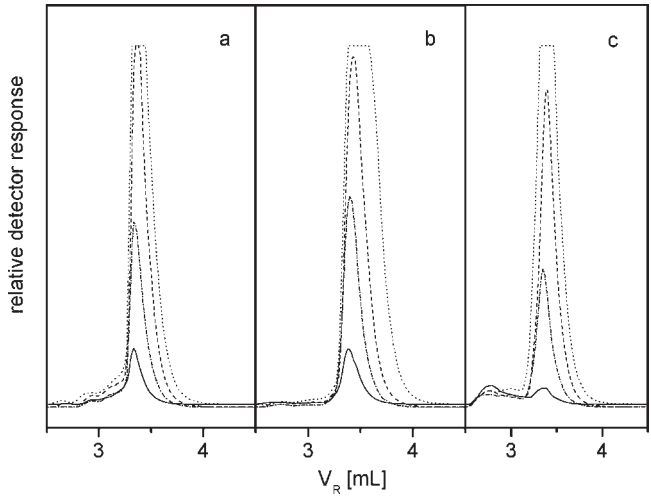

Figure 2.
The typical LC LCI chromatograms for a set of two monoliths monitored with toluene/methanol eluent containing 85 wt. % of toluene and with 100 μL barrier of neat methanol. Injected volumes v_i were (—) 20 μL; (-.-.-) 50 μL; (----) 100 μL and (.. . ..) 200 μL. Molar masses of polystyrene (a) 9,000; (b) 498,000; (c) broad molar mass sample with $\overline{M}_w = 180{,}000$ and $\overline{M}_n = 67{,}000$ – all data are in $g \cdot mol^{-1}$.

methanol or a toluene/methanol mixture containing 90 wt. % of methanol. Similar experimental conditions were applied also in present study.

Typical LC LCI chromatograms are displayed in Figure 2 for narrow polystyrenes with $M = 9$ and 498 $kg \cdot mol^{-1}$, as well as for the broad molar mass distribution sample with above eluent and the barrier of neat methanol. Methanol efficiently blocked elution of PS between about 100 and 2,000 $kg \cdot mol^{-1}$ contained in PS 498 $kg \cdot mol^{-1}$ (determined by independent SEC measurements). However, it seems that a fraction of polystyrene with 9 $kg \cdot mol^{-1}$ as well as of broad PS partially broke-through the barrier. Probably the lowest molar masses present in the broad PS sample were no more efficiently hindered by the methanol barrier. The main peaks of polymers are narrow and their widths only little depend on the injected volume v_i up to 100 μL. The peak widths at $v_i = 200$ μL were still acceptable considering the total volume of mesopores in two monoliths only in the range of 1 mL.

Figure 3 shows the log M vs. V_R dependences for polystyrenes with the barrier of neat methanol. The LC LCI principle works well also with the monolithic columns. Fast elution of macromolecules is efficiently hindered with the nonsolvent barrier. As result, polymers elute irrespective of their molar mass. Surprisingly, the barrier of neat methanol seems to stop large part of polystyrene with the molar mass as low as 0.67 $kg \cdot mol^{-1}$. For comparison, the log M vs. V_R dependence for PS in pure toluene (cf. Figure 1) is also depicted in Figure 3. The reasons for a decrease of polystyrene retention volumes obtained in the mixed eluent without the barrier action are so far unknown. One can speculate that molecules of methanol preferentially solvate surface of silica monolith and thus decrease accessible volume of mesopores. Still, the explicit explanation of this phenomenon needs further experimental results.

The chromatograms produced by the excess of either methanol or toluene (30 μL in 1 mL of eluent) are shown in Figure 4. The sorption equilibrium of toluene/methanol 85/15 wt./wt. eluent on the silica surface was massively perturbed[49] by the pulses of solvents with composition strongly

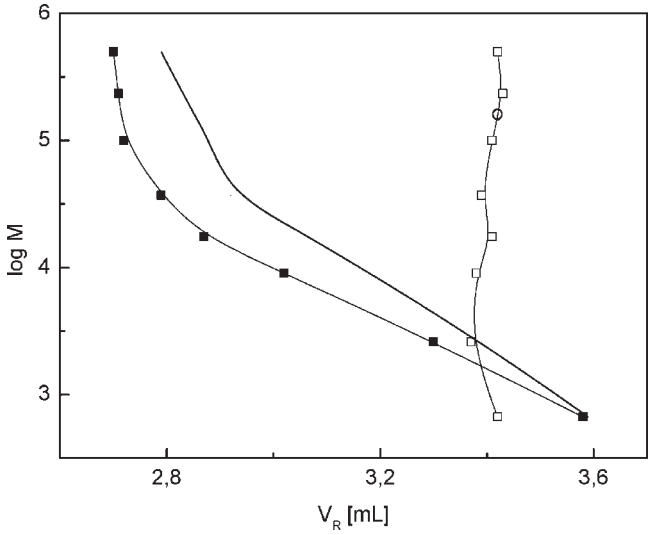

Figure 3.
The log M vs. V_R dependences for PS obtained with a set of two monoliths and eluent toluene/methanol containing 85 wt. % of toluene. PS was dissolved and injected in eluent without (■) and with (□) a 100 μL barrier of neat methanol. For comparison the log M vs. V_R dependence is depicted (—) for polystyrenes eluted in pure toluene (cf. Figure 1). The datapoint (○) belongs to the broad polystyrene, with $\overline{M}_w = 180{,}000\ g \cdot mol^{-1}$.

different from the composition of eluent. The peak of toluene was extensively broadened and that of methanol was split. A relatively large portion of methanol eluted from monolith with the low V_R. It is likely that this part of methanol with low V_R acted as the actual barrier for polystyrenes. This is why the nearly vertical log M vs. V_R dependence obtained in the LC LCI mode intercepts the SEC one

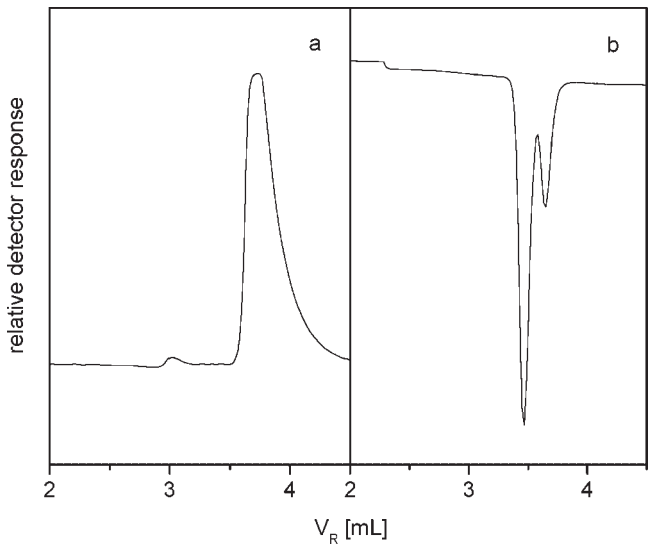

Figure 4.
The chromatograms of toluene (a) and methanol (b) excess (30 μL in 1 mL of eluent) injected into the tandem of two monoliths flushed with eluent toluene/methanol containing 85 wt. % of toluene.

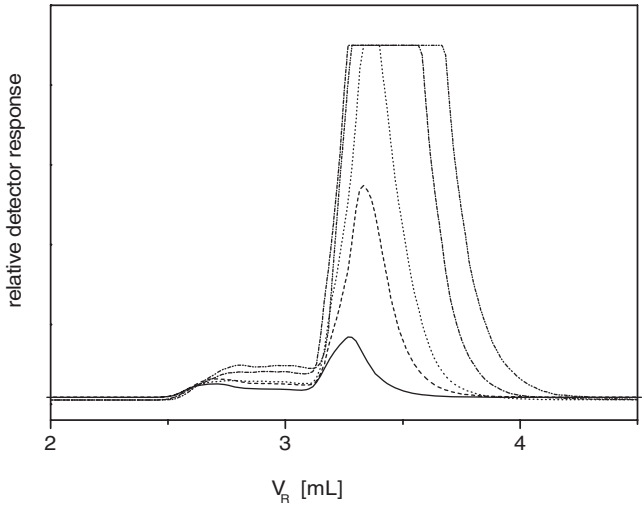

Figure 5.
The LC LCI chromatograms for various injected concentrations c_i of broad polystyrene with mixed eluent toluene/methanol containing 85 wt. % of toluene. Injected volume v_i was 20 μL, volume of barrier of neat methanol was 100 μL. Injected concentrations were: (—) 2; (---) 5; (. . ..) 10; (-.-.-) 25 and (-..-..-) 50 mg · mL^{-1}.

(Figure 3). Similar behavior was observed in some previously studied LC LCI systems, especially for the wide pore column packings.[13] Still, so far there is no explanation for the very low V_R of oligomeric polystyrene with $M = 0.67$ kg · mol^{-1}.

A set of chromatograms of broad PS injected at different concentrations is depicted in Figure 5. The result shows that a narrow 100 μL barrier of neat methanol rather efficiently decelerated fast progression of large part of polystyrene – even at its extremely high concentrations of 5 wt. %. Still, certain part of sample broke–thru the barrier, even at the very low injected polymer concentration (cf. also Figure 2). The Figure 5 again demonstrates the shortages of monoliths, namely presence of large mesopores and their low effective volume. Only about 0.7 mL remained for accommodation of peaks for non-retained macromolecules in the tandem of two monoliths. It means that a set of several monolithic columns would be needed for efficient preparative separation of two polymer species of different nature excluded from the mesopores of monoliths. This conclusion casts some doubt upon advantages of presently available monoliths against packed columns in the LC LCI applications.

Typical results obtained with a tandem of three monoliths are depicted in Figure 6 to 8. Eluents were mixtures of toluene and methanol containing 80 or 85 wt. % of toluene. The barrier was a mixture of toluene and methanol with 50 or 20 wt. % of toluene.

As shown also in[13] the barrier with lower concentration of methanol still blocked PS but it allowed break-thru of PMMA (Figure 6). This enabled efficient separation of PS and PMMA even if their molar masses were similar (Figure 7). However, for a high-speed base-line separation of these polymers four or five monolithic columns would be needed. The base line separation of narrow PMMA (minor component, 1%) from the broad PS (major component, 99%) was not attained (Figure 8), also due to extensive break-thru of broad PS (cf. Figure 2c).

In this series of experiments, the molar mass range of the LC LC eluted PS was extended up to 30,000 kg · mol^{-1} (Figure 6 and 9). It is evident that the LC LCI procedure does not exhibit upper molar

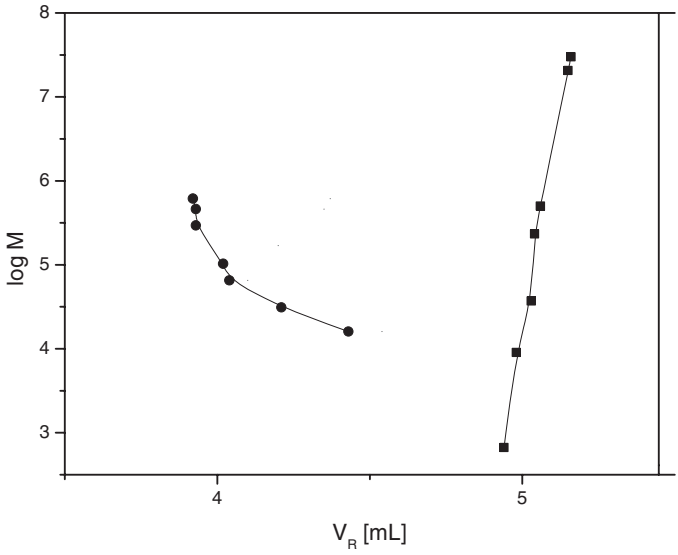

Figure 6.
The log M vs. V_R dependences for polystyrenes and poly(methyl methacrylate)s with a tandem of three monoliths and mixed eluent toluene/methanol containing 80 wt. % of toluene. Injected volume and concentrations of sample were 50 μL and 1 mg · mL^{-1}, respectively. 100 μL barrier toluene/methanol contained 50 wt. % of toluene. (■) PS and (●) PMMA.

mass limit of polymer (Figure 9). On the other hand, extremely large macromolecules exhibited increased tendency for breaking thru the barrier (Figure 9). The tentative explanation of this phenomenon considered insufficient difference in elution rate of macromolecules and small molecules of barrier when pore sizes exceeded

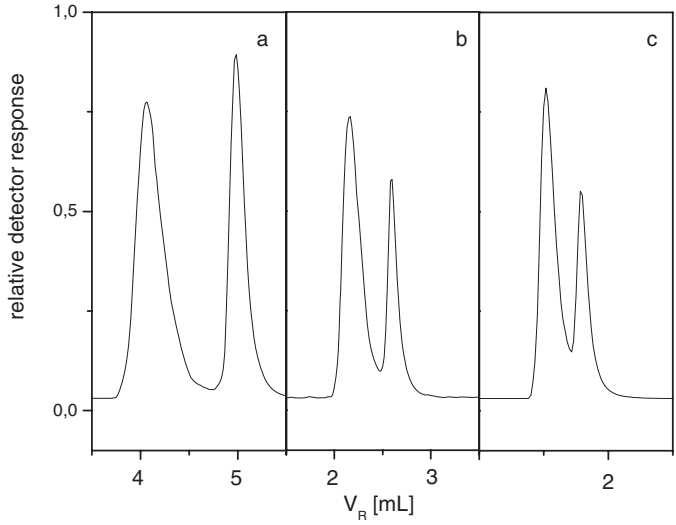

Figure 7.
The LC LCI chromatograms of a mixture of broad PS plus PMMA with molar mass 103,000 g · mol^{-1}, 1 mg · mL^{-1} each. Injected volume was 50 μL. Tandem of three monoliths, eluent was toluene/methanol containing 85 wt. % of toluene. 100 μL barrier of toluene/methanol mixture contained 20 wt. % of toluene. Flow rates: (a) 1 mL · min^{-1}; (b) 2 mL · min^{-1}; (c) 3 mL · min^{-1}.

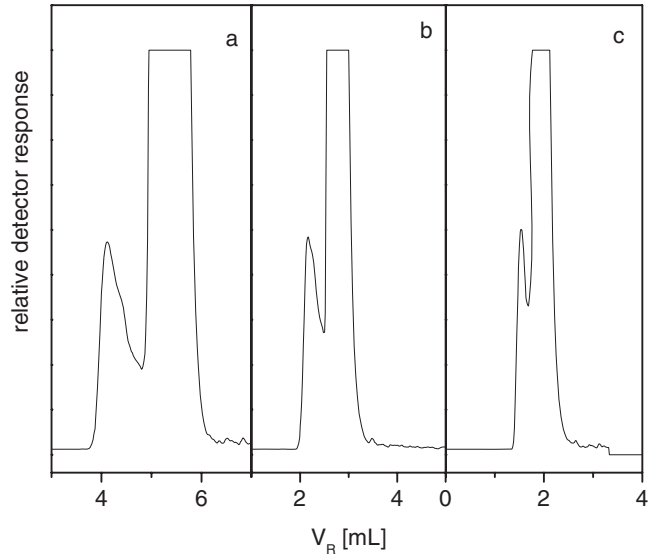

Figure 8.
The LC LCI chromatograms of a mixture of broad PS plus PMMA with molar mass 103,000 $g \cdot mol^{-1}$. Injected solution contained 0.1 g of PS and 1 mg of PMMA in 1 mL. Injected volume 50 μL. Eluent toluene/methanol contained 85 wt. % of toluene. 100 μL barrier of toluene/methanol contained 20 wt. % of toluene. Flow rates: (a) 1 $mL \cdot min^{-1}$; (b) 2 $mL \cdot min^{-1}$; (c) 3 $mL \cdot min^{-1}$.

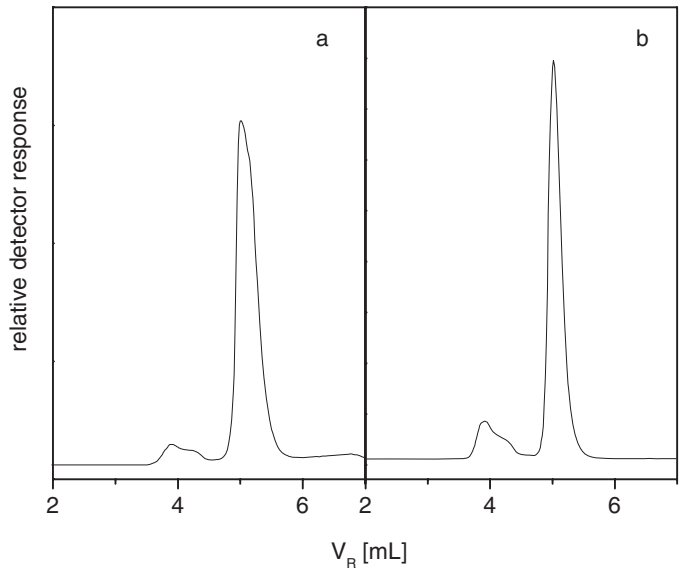

Figure 9.
The LC LCI chromatograms of ultra-high molar mass polystyrenes with M: (a) 20,600,000 and (b) 30,000,000 $g \cdot mol^{-1}$. Eluent was a mixture of toluene/methanol containing 85 wt. % of toluene. Sample volume v_i and concentration c_i were 50 μL and 1 $mg \cdot mL^{-1}$, respectively. 100 μL barrier of toluene/methanol contained 20 wt. % of toluene.

certain limit. The largest mesopores and the smallest macropores present in monoliths may be responsible for this kind of the break-thru phenomena. Important peak splitting was found in LC LCI when applying wide pore particulate column packing.[13] Alternatively, formation of the associates or even the microphases of LC LCI eluted macromolecules in contact with the precipitant on the barrier edge may contribute to the irregularities in the sample elution. The very large associates can probably break-thru the barrier to be freely eluted from the monoliths. The extent of association and microphase formation depends on the thermodynamic quality of the barrier toward macromolecules. The higher polymer molar mass and the more nonsolvent in the barrier zone the larger tendency to phase separation and the more intensive break-thru phenomenon is expected.

In order to test this hypothesis, both the broad and the ultra-high molar mass PS were injected into the eluent containing 85 wt. % of toluene behind barriers containing decreasing concentration of methanol. The results are shown in Figure 10 and 11. Evidently the extent of break-thru phenomenon of very large macromolecules drops with the decreasing content of methanol in barrier (Figure 10). However, if barrier becomes inefficient for the lowest molar masses (e.g. for those present in the broad PS) the break-thru appears again (Figure 11). These results show that optimization of experimental conditions is needed to suppress breaking-thru both very small and very big polymer species in LC LCI. Let us add that so far we have not observed the intensive break thru phenomenon in LC LC procedures based on the adsorption or enthalpic partition retention mechanisms. All these facts indirectly support the hypothesis on the role of associates and/or microphases in the break-thru processes. A more detailed study of the break-thru phenomena in the LC LC methods is under preparation.

For comparison, some LC LCS experiments were performed with monolithic columns. As mentioned in the Introduction, in this case eluent is a weak nonsolvent for polymer, which is injected in a good solvent.

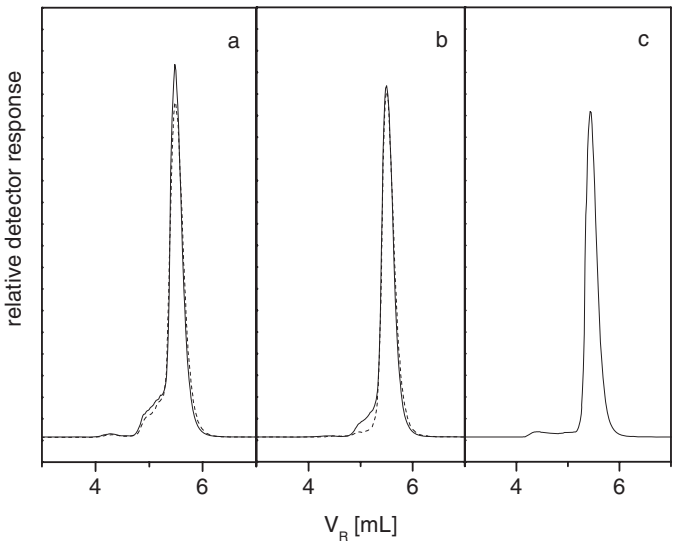

Figure 10.
The LC LCI chromatograms of ultra-high molar mass polystyrene with M 30,000,000 $g \cdot mol^{-1}$. Eluent was a mixture of toluene/methanol containing 85 wt. % of toluene. Sample volume v_i and sample concentration c_i were 50 μL and 1 $mg \cdot mL^{-1}$, respectively. 500 μL barriers of toluene/methanol contained (a) (—) 30; (---) 40; (b) (—) 50; (---) 60 and (c) (—) 70 wt. % of toluene.

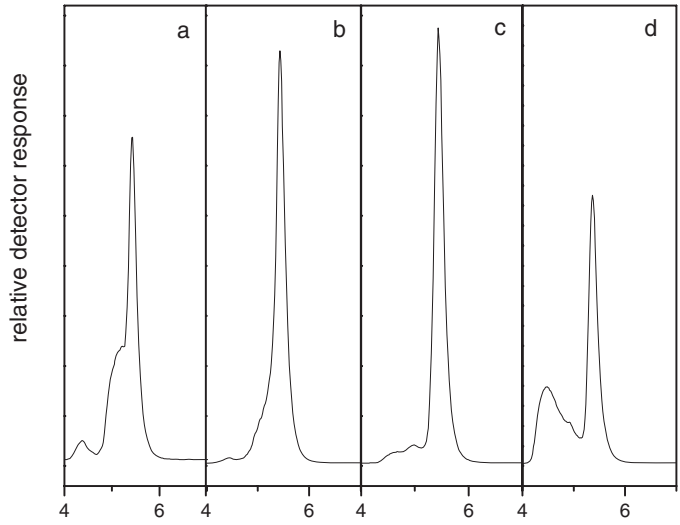

Figure 11.
The LC LCI chromatograms of broad polystyrene. Eluent was a mixture of toluene/methanol containing 85 wt. % of toluene. Sample volume v_i and sample concentration c_i were 50 μL and 1 mg · mL^{-1}, respectively. The barriers of toluene and methanol were applied containing: (a) 30; (b) 50; (c) 60 and (d) 70 wt. % of toluene.

Problems with the sample break-thru are reduced in this arrangement as the barrier is continuous. On the other hand, the experimental flexibility of work with the narrow barrier and even with tandem of several barriers is lost. Typical LC LCS results are shown in Figure 12 and 13. presence of PS (result not shown). Figure 12 demonstrates

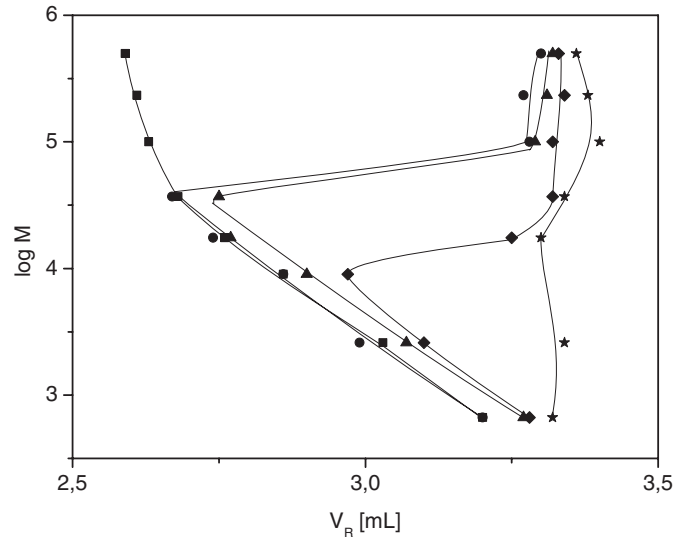

Figure 12.
The log M vs. V_R dependences for polystyrenes dissolved and injected in pure toluene into a tandem of two monoliths columns. Injected volume v_i and injected concentration c_i were 50 μL and 1 mg · mL^{-1}, respectively. Eluents were mixtures of toluene/methanol containing (□) 80; (●) 70; (▲) 65; (◆) 57; and (★) 54 wt. % of toluene.

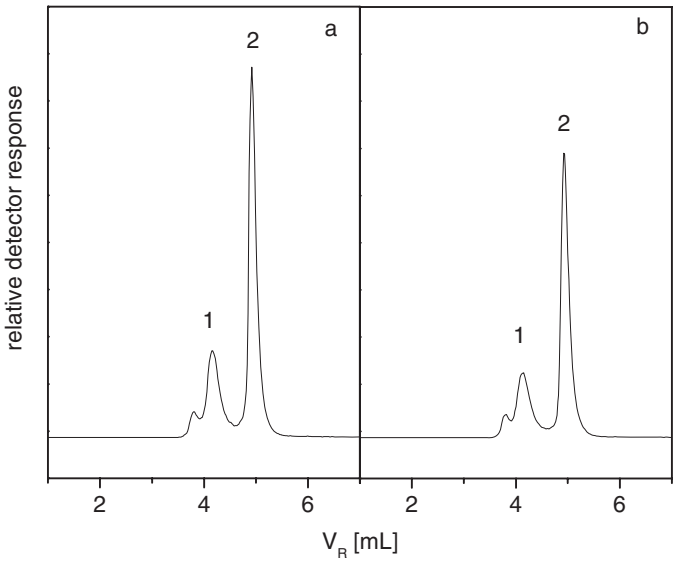

Figure 13.
Examples of polystyrene and poly(vinyl acetate) separation applying the LC LCS procedure. Tandem of three monoliths was applied with mixed eluent toluene/methanol containing 50 wt. % of toluene. Injected volume v_i and sample concentration c_i were 50 μL and 1 mg · mL^{-1}, respectively. Samples were dissolved and injected in pure toluene. Broad PVAC was separated from PS with molar mass (a) 233,000 and (b) 498,000 g · mol^{-1}. (1) Broad PVAC; (2) PS.

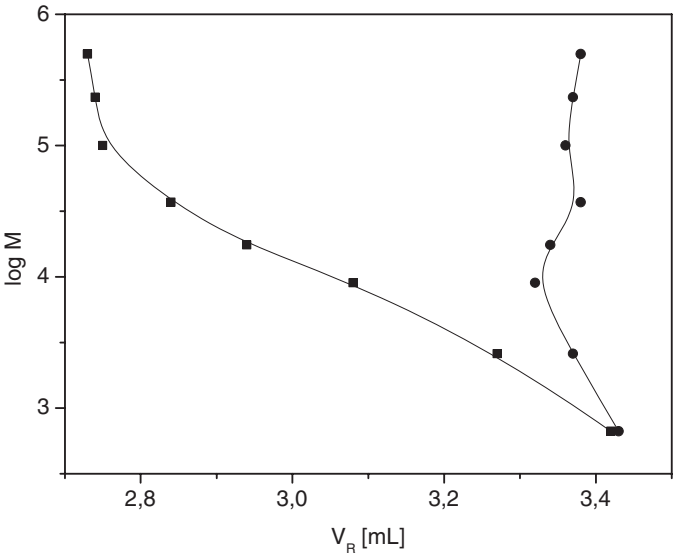

Figure 14.
The log M vs. V_R dependences for polystyrenes with a tandem of two monoliths and mixed eluent toluene/acetonitrile containing 50 wt. % of toluene. Sample volume v_i and sample concentration c_i were 20 μL and 1 mg · mL^{-1}, respectively. (■) sample without barrier (●) sample with a 100 μL barrier of neat ACN.

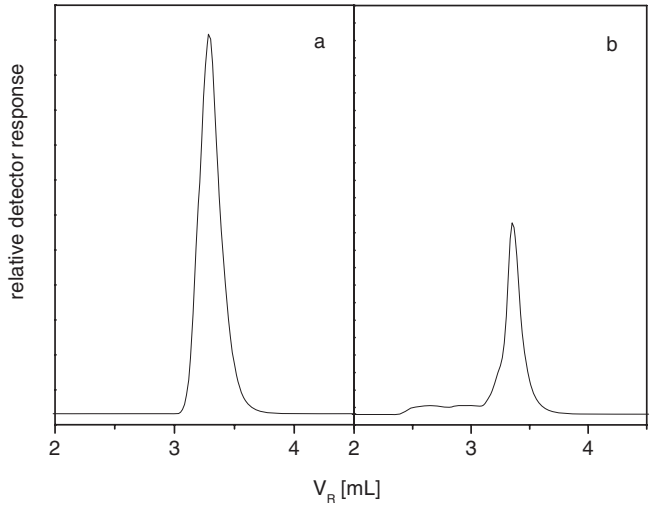

Figure 15.
The LC LCI chromatograms of polystyrenes eluted from a tandem of two monoliths. Eluent was a mixture of toluene/ACN containing 50 wt. % of toluene. Sample concentration c_i and sample volume v_i was 1 mg · mL^{-1} and 20 μL, respectively. Barrier was 100 μL of neat ACN. Molar masses of PS: (a) 2,600 and (b) 498,000 g · mol^{-1}.

how the deteriorating eluent quality for the polymer sample promotes transition from the SEC to the LC LCS elution mode. An efficient LC LCS separation of PVAC from PS is depicted in Figure 13. However, so far the reason is unknown for the peak splitting of broad PVAC, which elutes in the exclusion mode. The same shape of PVAC chromatograms was observed also without presence of polystyrene in the sample.

In the next series of LC LCI experiments, mixed eluent toluene/acetonitrile containing 50 wt. % of toluene was applied. It well dissolved both PS and PMMA. Barrier liquid was ACN, which is a precipitant for PS. Selected results are depicted in Figure 14 and 15. The LC LCI principle was operative also in this system and PS was efficiently decelerated by the ACN barrier (Figure 14). However, again a non-negligible fraction of broad PS broke-through and eluted in form of the additional skewed peak (Figure 15). The break-through phenomenon is more pronounced than in case of toluene/methanol eluent with the barrier of neat methanol (compare Figure 2 and Figure 15). This result again shows limitations of the LC LCI procedure with the wide-pore column filling.[13]

Conclusions

It has been shown that the barrier mechanism of liquid chromatography under limiting conditions (LC LC) of polymer solubility and insolubility is operative also with the monolithic HPLC columns. Outstanding permeability of silica based monoliths enables application of both high eluent flow rates and sample concentrations. Polymers with extremely, high molar masses can be easily processed, as well. However, the tested monoliths exhibit relatively low volume and large size of mesopores. These properties of monoliths to some extent reduce their applicability in the solubility based LC LC procedures. The break-thru phenomena were observed. It is likely that both the lowest and the highest sample molar masses show the increased break-thru tendency, which can be suppressed by optimization of experimental conditions. However, this impairs important asset of

the LC LC procedures namely their robustness. With the presently available monoliths, the LC LC separations based on the nonsolvent barriers can be beneficially applied in the preparative purification of polymer species from the unwanted macromolecular admixtures of other nature, especially when the presence can be tolerated of a nonsolvent in the purified material. In order to take full advantage of monolithic columns in LC LC, volume of their mesopores should be raised and the size (effective diameters) of their mesopores should be reduced.

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